SUBSTITUTES FOR ABSOLUTE ETHYL ALCOHOL

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During the past year the writer has had his interest attracted to the question of whether other alcohols can be successfully substituted for anhydrous ("absolute") ethyl alcohol in histological work. For a considerable part of the year the air of Portland, Oregon, is nearly saturated with water vapor, and the tendency of absolute ethyl alcohol to absorb water constitutes one of the difficulties in its use. More potent reasons for seeking substitutes were, however, the high cost of the absolute ethyl alcohol and annoyances resulting from tax and prohibition laws. Even when alcohol can be secured by institutions free of tax there are regulations to be observed which entail a certain amount of delay in securing it, as well as much supervision of its use. So it would be advantageous if other alcohols can be used which are not subject to the regulations of the Bureau of Internal Revenue, and which are not sought as beverages. Fortunately, there are at least three such alcohols which have proved to possess merit.

METHYL ALCOHOL. The ordinary commercial quality of methyl alcohol contains about 95% of alcohol, the remainder consisting mostly of acetone, with traces of a large variety of other impurities. Purified methyl alcohol, anhydrous, and nearly free from acetone and other impurities, is put on the market under various trade names. The brand which we have tested is known as Diamond Methyl alcohol. Being practically free from acetone it not only lacks the strong disagreeable smell of ordinary wood alcohol but, in fact, has a pleasant odor much like that of refined grain alcohol. This alcohol dehydrates sections and tissues as well as the absolute ethyl alcohol, and is a little more reliable because it will dehydrate more sections than an equal amount of absolute grain alcohol. We have found it to be a good solvent, and have used it with success in the compounding of a number of reagents. As regards cost, it was not only far cheaper than anhydrous ethyl alcohol, but was considerably cheaper than the tax-free 95% grain alcohol which we bought a short time before we secured the methyl alcohol. We are now using this alcohol as our standard reagent in dehydration. In the course of eight months our stock, kept in a large glass stoppered bottle, has not absorbed enough water vapor to be noticeable. We purposely made no particular effort to seal the stock bottles tightly from the atmosphere. Anhydrous ethyl alcohol kept under the same conditions would have been useless for the dehydration of tissues.

BUTYL ALCOHOL. Professor George W. Martin has called attention (Science, April 21, 1922), to the use of butyl alcohol in dehydration and infiltration with parafin. This alcohol has been under test in our laboratory as a dehydrating reagent for several months and has given excellent results. We have used it, however, only in the last stage of dehydration, passing slides from 90% methyl or 95% ethyl alcohols to the butyl alcohol. It appears to us to be superior to either ethyl or methyl alcohols for dehydration, but its use is slightly disagreeable on account of the pungent, characteristic odor. Inhalation of its fumes causes a slight, temporary irritation of the throat. In reply to our inquiry as to whether this property of butyl alcohol might be removed, the Commerical Solvents Corporation, which made our sample, replied:

"The irritation of the throat, caused by the use of Butanol, is quite characteristic of this compound and is a property which it would be difficult to obliterate. However, we do not believe it has any harmful effect, as some of the men who work on the distillation end of the process have been subjected to this for years and have experienced no ill effects. They are much less susceptible to the irritation after having worked with this compound for some time."

Butyl alcohol is, at any rate, a valuable reagent for dehydration, our observation being that the sections dehydrated with it are slightly more brilliant than those cleared with the previously mentioned alcohols. As Professor Martin also states, butyl alcohol is a solvent of paraffin, and can be used for infiltration of tissues likely to shrink or harden in the usual infiltrants. We believe, however, that for this purpose it is surpassed by Terpeneol.

TERPENEOL. The use of terpeneol (terpineol) in place of absolute ethyl alcohol was suggested a number of years ago, but it is only lately that I have been able to test it thoroughly. Terpeneol is a pleasant smelling, aromatic liquid, of about the consistency of thin cedar oil. It is tolerant of large amounts of water in dehydration, and also dissolves paraffin and resins. On account of its consistency we have found it advisable to use first a mixture of terpeneol and methyl alcohol before placing tissues into pure terpeneol. Terpeneol may be used as a dehydrating agent for sections, but does not have any advantages over methyl or butyl alcohol when used in that way. As it dissolves parafin readily it is more useful as a dehydrant and infiltrant of tissues to be embedded. Terpeneol dissolves parafin better than butyl alcohol. Our experience has been that tissues which had been dehydrated and infiltrated with terpeneol were less shrunk and hardened than when embedded by the ordinary methods. Terpeneol is of rather high refractive index, so that it serves as a clearing agent also. Sections may be transferred directly from terpeneol to Xylol-damar. As the terpeneol

does not make tissues so brittle as does Xylol it can be used advantageously in the preparation of whole mounts.

We have also found that damar dissolved in terpeneol makes a mounting medium which, on account of the refractive index being lower than that of xylol-damar or xylol-balsam, shows some details of cell structure which are obscured in these commonly used mounting media.